



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

6-27-89
WSA Helt
8/8

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review of Oxyfluorfen (Goal)

FROM: Kerry L. Dearfield, Ph.D. *Kerry L. Dearfield*
Executive Secretary, Peer Review Committee 6.27.89
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: Lawrence Schnaubelt
Product Manager Team #23
Registration Division (H7505C)

The Health Effects Division Peer Review Committee met on 5/24/89 to discuss and evaluate the weight-of-the-evidence on Oxyfluorfen with particular reference to its oncogenic potential. The Committee unanimously classified Oxyfluorfen as a Category C - Possible Human Carcinogen based on hepatocellular adenomas and carcinomas in male CD-1 mice. Quantification of oncogenicity risk was not recommended at this time.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

William L. Burnam

Reto Engler

Edwin R. Budd

Marcia Van Gemert

John Quest

Esther Rinde

Kerry Dearfield

Lynnard Slaughter

W. L. Burnam
Reto Engler
Edwin R. Budd
Marcia Van Gemert
John A. Quest
Esther Rinde
Kerry L. Dearfield
L. J. Slaughter

Marion Copley

Marion Copley 7/7/89

George Ghali

G. Ghali

Karl Baetcke

Karl Baetcke

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

William Dykstra

William M. Dykstra

Robert Zendzian

Robert Zendzian 7/5/89

Bernice Fisher

Bernice Fisher

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Penelope A. Fenner-Crisp

Penelope A. Fenner-Crisp

Richard Levy

Richard Levy

William Sette

William Sette

Richard Hill

Robert Beliles

Robert Beliles

4. Other Attendees:

Karen Hamernik (HED)

Hugh Pettigrew (HED)

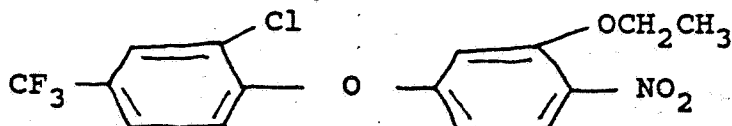
B. Material Reviewed:

The material available for review consisted of i) reviews for a mouse oncogenicity study and a rat oncogenicity study, ii) a CAG review of these same mouse and rat oncogenicity studies, iii) one-liners, and iv) other data summaries prepared by William Dykstra; v) tables and statistical analysis were prepared by Bernice Fisher. The material reviewed is attached to the file copy of this report.

C. Background Information: Oxyfluorfen is an unconditionally registered herbicide with tolerances established in Section 180.381 of Title 40 of the Code of Federal Regulations for a variety of raw agricultural commodities (RACs). The herbicide is also known as Goal and RH-2915. The registrant is Rohm and Haas Company. The chemical name is 2-chloro-4-trifluoromethyl-3'-ethoxy-4'-nitrodiphenylether.

The Chemical Abstracts Number is 42874-03-3 and the Tox Chem Number is 188AAA.

Structure of Oxyfluorfen:



D. Evaluation of Oncogenicity Evidence for Oxyfluorfen:

1. Mouse Oncogenicity Study

Reference: Goldenthal, E.I. April 25, 1977. Twenty month dietary feeding study in mice. Unpublished report No. 285-012 prepared by International Research and Development Corporation. Submitted by Rohm and Haas Company, Philadelphia, PA. Accession No. 37939.

Oxyfluorfen (85.7% a.i.) was administered in the diet to groups of 60 male and 60 female Charles River CD-1 mice at levels of 0, 0 (ethanol), 2, 20 and 200 ppm for 20 months. It is not clear why the ethanol control was used as ethanol was not a vehicle for the test groups. An interim sacrifice was made at 52 weeks with 5 animals in dose levels of 0, 0 (ethanol) and 200 ppm. The 2 ppm dosed animals were sacrificed at 18 months instead of 20 months. It is noted that animals in the 200 ppm dose group were dosed at 800 ppm for 2 weeks (weeks 57 and 58) during the study.

Compared to either control (untreated and ethanol), liver adenoma, carcinoma and combined adenoma and/or carcinomas in male mice exhibited significant increasing trends with dose increments of oxyfluorfen (Table 1). The pairwise comparison of ethanol control with the highest dose group was significantly different from combined adenomas and/or carcinomas in male mice. Female mice did not manifest any significant tumorigenicity responses.

Table 1. Oxyfluorfen, Mouse Study - Male liver tumor (adenoma and carcinoma rates⁺ and Cochran-Armitage trend test and Fisher exact test results (p values))

<u>Liver Tumor</u>	<u>Dose level (ppm)</u>				
	0 untreated	0 ethanol	2 ^a	20	200 ^b
<u>Adenoma</u> (percent)	1/47 (2)	0/47 (0)	0/44 (0)	1/44 (2)	3/52 (6) ^c
untreated 0, p=	0.0472*	--	0.5165	0.7360	0.3478
ethanol 0, p=	--	0.0133*	1.0000	0.4835	0.1409
<u>Carcinoma</u> (percent)	1/47 (2)	1/47 (2)	0/44 (0)	3/44 ^d (7)	5/52 (10)
untreated 0, p=	0.0205*	--	0.5165	0.2837	0.1272
ethanol 0, p=	--	0.0205*	0.5165	0.2837	0.1272
<u>Both</u> (percent)	2/47 (4)	1/47 (2)	0/44 (0)	4/44 (9)	8/52 (15)
untreated 0, p=	0.0039**	--	0.2640	0.3073	0.0643
ethanol 0, p=	--	0.0017**	0.5165	0.1606	0.0226*

⁺ Tumor bearing animals / number of animals examined, excluding those that died before week 53.

^a animals at 2 ppm were sacrificed 10 weeks earlier (week 77) than all others.

^b animals dosed at 800 ppm for weeks (57 and 58).

^c first adenoma observed at week 87.

^d first carcinoma observed at week 61.

Note: Significance of trend test denoted at Control.

Significance of pairwise comparison with control denoted at Dose level.

** p < 0.01 and * p < 0.05

a. Discussion of Tumor Data

Historically, these exposed animals have been examined by a number of parties. The original report by IRDC suggested a dose related trend in hepatocellular carcinomas for male mice. There was a slight increase in hepatocellular carcinomas noted at 200 ppm, but the increase was not significant by pairwise comparison to controls. There was also a finding of hepatocellular regeneration reported. To examine the nature of the regenerative changes, Dr. Paul Newberne of the Department of Nutrition and Food Science, Massachusetts Institute of Technology was asked to review the livers of mice that died or were sacrificed during the second year of the study. He reported nuclear and cellular enlargement, nuclear abnormalities and mild necrosis. Though he did not find a statistically significant increase in tumor incidence, there was a statistically significant increase by pairwise comparison in the incidence of "nodules of atypical hepatocytes" (hyperplastic nodules - current thinking considers these to be adenomas) at the 200 ppm dose in male mice. A subsequent evaluation ensued performed by Drs. Robert Squire and Bernard Haberman as part of the Cancer Assessment Group's (CAG's) assessment of Oxyfluorfen. They found a statistically significant dose related trend for combined adenomas and/or carcinomas. No significant pairwise comparisons were found. The data from the CAG evaluation were used to perform the statistical analyses found in Table 1.

Oxyfluorfen was associated with a significant positive dose related trends for male hepatocellular adenoma, carcinoma and combined adenoma and/or carcinoma. Historical control data from IRDC for CD-1 mouse studies of 20-22 month duration during the period of 1973 to 1978 (7 studies) indicate the following ranges for males: 0-4% adenomas, 0-6% carcinomas and 0-10% combined (assumed adenoma and carcinoma numbers are from different animals for the combined value). For adenomas, the 200 ppm value (6%) is above the historical range; for carcinomas, the 20 ppm (7%) and 200 ppm (10%) values are above the historical range; and for combined tumors, the 200 ppm value (15%) is above the historical range. The incidence in control males of the Oxyfluorfen study is 2/47 (4%) and is within the range of historical controls reported. There was no apparent effect on the latency period for tumor occurrence. No compound related increase in tumors was observed in female mice.

b. Consideration of Adequate Dosing for Assessment of Oncogenic Potential

In this study, significant decreasing mortality trends with incremental doses of Oxyfluorfen were found with the use of either of the two control groups. No other significant differences were found in the mortality statistics. Changes in body weight were similar for control and treated mice. Food consumption was similar for control and treated mice. Little compound related effects were

found for toxic signs, hematology or biochemical parameters. There was a 17-34% increase in group mean relative liver weight in the 200 ppm males at 1 year. At the terminal sacrifice, there was a 28-43% increase in group mean relative liver weight in the 200 ppm males which was also accompanied by hepatocellular lesions including necrosis. The number of animals with hepatocellular cytomegaly, karomegaly and/or single cell necrosis (S.C.N.) is shown in Table 2. There were no compound related instances of focal or massive necrosis, fibrosis or inflammation associated with exposure to Oxyfluorfen.

Table 2. Mouse 20 Month Study - Incidence of Non-Neoplastic Liver Lesions

<u>Males</u>					
	<u>Untreated Control</u>	<u>Ethanol Control</u>	<u>2 ppm</u>	<u>20 ppm</u>	<u>200 ppm</u>
Cytomegaly, Karomegaly, and S.C.N.	0/49	17/47	17/47	25/46	42/55
S.C.N. alone	0/49	2/47	3/47	5/46	27/55
<u>Females</u>					
Cytomegaly, Karomegaly, and S.C.N.	1/51	7/48	8/49	7/48	26/52
S.C.N. alone	1/51	3/48	5/49	3/48	16/52

The Peer Review Committee agreed that the signs observed in the chronic mouse oncogenicity study were not sufficient enough for adequate maximal dosing to fully assess oncogenic potential. The single cell necrosis in the long term study was particularly examined, but finally considered as not life threatening (decreased mortality trend) and 200 ppm was not an adequate top dose. The registrant performed a 90-day study subsequent to this oncogenicity study to more fully evaluate the dose selection for the long term study.

Oxyfluorfen (72.5% a.i.) was given in the diet to CD-1 mice at doses of 0, 200, 800 and 3200 ppm. There were deaths related to treatment at the highest dose, 67% males died and 13% females died. A 7% mortality at 800 ppm in males was also seen; 0% for females. There were no decreases in body weight gain observed at any dose. Other signs included increases in platelets at all doses, decreases in hemoglobin at all doses, increases in SGPT in

males at 800 and 3200 ppm and in females at all doses, and increased liver weight in males and females at all doses. Specifically in the liver, an increased incidence of diffuse hepatocytic hypertrophy in all males and females, increased incidence of single cell necrosis in all males at 800 and 3200 ppm and focal necrosis in males at 200, 800 and 3200 ppm were observed.

Based on the results seen in both the long term oncogenicity study and the 90-day study on CD-1 mice, the Peer Review Committee considered that the 200 ppm dose was not a high enough dose to fully assess the oncogenic potential of Oxyfluorfen. However, it was felt that an 800 ppm dose (or one slightly lower) may have been a dose that could have approached or approximated an adequate top dose for a long term oncogenicity study.

2. Rat Oncogenicity Study

Reference: Rinehart, W.E. January 1, 1978. A twenty-four month oral toxicity/carcinogenicity study of RH-2915 in rats. Unpublished report No. 75-1111A prepared by Bio/dynamics, Inc.; histopathology by Research Pathology Services, Inc. Submitted by Rohm and Haas Company, Philadelphia, PA. Accession Nos. 83445 and 096872.

Oxyfluorfen (85.7% a.i.) was administered in the diet to groups of 50 male and 50 female Long-Evans rats at "specific dose levels" for 24 months. Initial dose levels were gradually increased over a 4 week period in order to acclimate the animals to compound consumption. At week 5, dose levels were established at 0, 2, 40 and 800 ppm. Through a calculation error, animals in the 800 ppm group actually received 685 ppm a.i. in the diet for weeks 5 through 48; upon discovery of the error, dose correction to 800 ppm was made. At week 57, the 800 ppm dose was increased to 1600 ppm in an attempt to establish an "effect" level. An interim sacrifice at 12 months was performed with 5 animals/sex (control) and 10 animals/sex (high dose).

Under the conditions of the study, Oxyfluorfen did not produce evidence of compound related increased incidences of any tumor types examined. There was no effect on latency.

Consideration of Adequate Dosing for Assessment of Oncogenic Potential

The Peer Review Committee concurred that there was no evidence of oncogenicity due to Oxyfluorfen in this Long-Evans rat study; however, there was a question whether there was adequate high dosing for assessment of oncogenic potential in this study. There were no compound related effects on mortality, toxic signs, ophthalmology, body weight, food consumption, hematology, clinical chemistries, urinalysis, and organ weights. At the 1-year interim

sacrifice, histopathologic liver lesions were observed in 7/10 high dose females and 1/10 high dose males. The liver changes consisted of a slight enlargement of centrilobular hepatocytes with slightly increased binucleation and nuclear enlargement. Peripheral lobular hepatocytes in some cases were minimally compressed. The 24-month histopathological examination showed one male and two females of the high dose with minimal hypertrophy of centrilobular hepatocytes of the liver. The Peer Review Committee did not consider these minimal signs to be significant enough for appropriate dose selection to properly assess oncogenic potential. Based on these data, this rat study is considered inadequate.

A 90-day study with Long-Evans rats was performed subsequent to the oncogenicity study in order to estimate the appropriate level of maximum dosing for the oncogenicity study. Oxyfluorfen (72.5% a.i.) was given in the diet at 0, 800, 1600 and 3200 ppm. There was little effect on mortality until the 3200 ppm dose where 10 males (66% of number on test) and 2 females (13%) died. Body weight gain decreases were reported: at 800 ppm, 6% in males and 0% in females; at 1600 ppm, 13% in males and 3% in females; and at 3200 ppm, 21% in males and 7% in females. There were dose related effects on several tissue/organ systems, including liver, adrenals, kidneys, spleen and bone marrow. These effects consisted primarily of hypertrophy or hyperplasia, in many instances found only at the top two doses. Diffuse hepatocellular hypertrophy was the predominant liver change. Centrilobular hepatic necrosis was observed in 3 high dose males with vacuolization seen in one of these. These liver effects appear consistent with those in the oncogenicity study. Based on the effects seen with this 90-day study, it was concluded that 800 ppm would not be a high enough dose to properly assess oncogenic potential. This supports the consideration that dosing could have been higher in the oncogenicity study.

E. Additional Toxicology Data on:

1. Metabolism

In rodents, the parent compound is found to be the largest component of fecal residues. In the male rat, essentially all associated radioactivity is found in the feces. In the female rat, approximately 5% of the radioactivity is found in the urine.

2. Mutagenicity

Four acceptable mutagenicity tests have been performed and submitted with Oxyfluorfen as the test substance. These studies indicate that technical grade Oxyfluorfen has mutagenic activity. They minimally satisfy the requirement for testing in the three categories of mutagenicity testing as follows:

Gene mutation assays:

a. Salmonella assay (Document #001854): technical grade (72.5% a.i.) material produced positive results in strains TA98, TA100 and TA1537 with and without activation. Positive results with activation were found at lower concentrations indicating that activation plays a role in the genetic activity of this compound. A polar fraction prepared from the same lot as the technical was also positive with activation in strain TA98 (only strain tested with polar fraction). However, a purified sample (99.7% a.i.) was found negative with and without activation in strains TA98, TA100, TA1535 and TA1537 at concentrations up to 7500 ug/plate.

b. Mouse lymphoma assay (Document #s 001854, 002166, 002414): technical grade material produced positive results with activation to levels 2 to 4 times background at concentrations up to 40 ug/ml with adequate toxicities. It was negative without activation to 1000 ug/ml (8% relative growth). A purified sample was found negative.

Structural chromosomal assays:

a. Rat bone marrow aberrations assay (Document #001854): single oral or 5 day oral dosing up to 1.19 g/kg/dose (some mortality at top dose) had no effect on the incidence of chromosomal aberrations in bone marrow of male rats.

Other genotoxic effects:

a. Unscheduled DNA synthesis (UDS) in primary rat hepatocytes (Document #001854): technical grade and a polar fraction of the technical grade did not induce UDS at concentrations up to 25 ug/ml.

In Document #004288, other mutagenicity studies are referred to, but no classifications were provided due to lack of details. Negative results were reported for a Salmonella assay and an assay using Saccharomyces as a tester organism. A host-mediated assay with Salmonella strains TA1530 and G46 was reported negative. Another assay with Saccharomyces cerevisiae strain D3 was reported to have a slightly increased recombinant frequency when tested in vitro, but was negative in a host-mediated assay.

Oxyfluorfen was originally placed into special review due to the presence of small amounts of perchloroethylene in the technical. A preliminary review of open literature suggests that perchloroethylene (tetrachloroethylene, CAS #127-18-4) does not produce mutagenic effects in Salmonella, mouse lymphoma, Drosophila sex-linked recessive, in vitro cytogenetic and in vitro sister chromatid exchange assays. It appears then that the mutagenic effects produced by oxyfluorfen may not be mediated by perchloroethylene.

3. Developmental and Reproductive Effects

Oxyfluorfen was assayed for potential developmental and reproductive effects in several tests. The evidence suggests that oxyfluorfen is capable of inducing developmental and reproductive effects.

a. Rat developmental assay (Document #004288): rats were exposed to levels of 0, 10, 100 and 1000 mg/kg. Developmental effects were observed at 1000 mg/kg as evidenced by a lower implantation efficiency, a higher resorption index and a lower fetal viability incidence. The fetotoxic NOEL was 100 mg/kg.

b. Rabbit developmental assay (Document #001572): NZW rabbits were exposed by gavage to levels of 0, 10, 30 and 90 mg/kg (top dose could not be evaluated). The developmental effect observed at 30 mg/kg was an increase in fused sternebrae. The maternal NOEL was 10 mg/kg and the LEL was 30 mg/kg (anorexia, decreased body weight gain). The fetotoxic NOEL was 10 mg/kg.

c. Mouse teratology assay ("Chernoff Screen") (personal communication to W. Dykstra from N. Chernoff): there is no formal assessment for this assay. The results are reported negative with oxyfluorfen.

d. 3-Generation rat reproduction study (Document #004288): rats were exposed to levels of 0, 2, 10 and 100 ppm. Effects were observed at 100 ppm as evidenced by decreases in fetal viability, fetal body weight and maternal body weight. The NOEL was 10 ppm.

4. Structure-Activity Correlations

Oxyfluorfen is structurally related to four other diphenyl ether herbicides that have oncogenicity evidence associated with them. These chemicals include lactofen, acifluorfen (Blazer, Tackle), nitrofen and fomesafen. The structures of these compounds are shown in Figure 1.

1. Lactofen produces hepatocellular carcinomas in male CD-1 mice at 250 ppm (HDT), combined hepatocellular carcinomas/adenomas in both sexes of mice at 250 ppm, and liver neoplastic nodules at 2000

ppm in both sexes of Sprague-Dawley rats. The Peer Review Committee classified lactofen as a B₂ oncogen. Lactofen was positive in the Salmonella assay (strain TA1538 without activation at ≥ 5000 ug/plate), but was negative for gene mutations and aberrations in cultured Chinese hamster ovary (CHO) cells and for UDS in primary rat hepatocytes.

2. Acifluorfen is the major metabolite of lactofen. Acifluorfen produces stomach papillomas (at 2500 ppm) and hepatocellular adenomas and carcinomas (625 and 2500 ppm - males; 2500 ppm - females) in both sexes of mice, but is negative in rats. The Peer Review Committee classified acifluorfen as a B₂ oncogen. Acifluorfen was positive for dominant lethal mutations and Y chromosome loss in Drosophila and for mitotic recombination in Saccharomyces. Marginal activity was found in a rat dominant lethal assay. Acifluorfen was negative in mouse lymphoma, rat bone marrow cytogenetics, UDS/primary rat hepatocyte and Drosophila sex-linked recessive lethal assays.

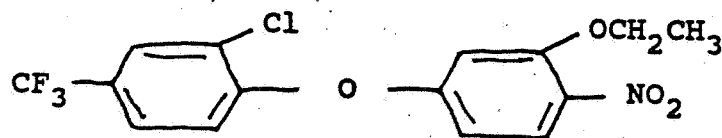
3. Nitrofen produces hepatocellular carcinomas at 2348 ppm (LDT) and hemangiosarcomas of liver at 4696 ppm (HDT) in both sexes of B6C3F1 mice. Pancreatic carcinomas were found in female Osborne-Mendel rats at 2600 ppm (HDT). Although this chemical has not been formally classified by the Peer Review Committee, during the discussion on oxyfluorfen, it was suggested that nitrofen most likely would be classified as a B₂ oncogen. Nitrofen has been found to be positive in the Salmonella assay, usually with activation, in many studies.

4. Fomesafen produces hepatocellular adenomas in male mice at 1, 100 and 1000 ppm and in females at 100 and 1000 ppm. Increased incidences of hepatocellular carcinomas and combined carcinomas and adenomas were found in both sexes at 1000 ppm (HDT). Negative results were obtained in a rat study. The Peer Review Committee classified fomesafen as a category C oncogen and determined that quantification of risk was appropriate. Fomesafen was found positive in a rat bone marrow cytogenetics assay, but negative in the Salmonella, mouse lymphoma, mouse micronucleus, UDS/cultured human cells and mouse dominant lethal assays.

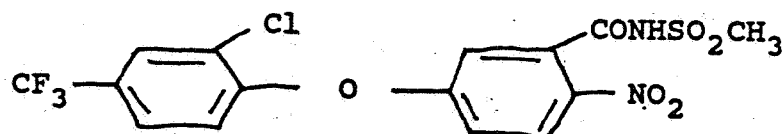
Lactofen and fomesafen would be expected to be metabolized through hydrolytic cleavage of the ester or amide linkage, respectively, to acifluorfen. The oxidative deethylation of oxyfluorfen would result in the hydroxy analogue of acifluorfen.

Figure 1: Structures of diphenyl ether herbicide analogues

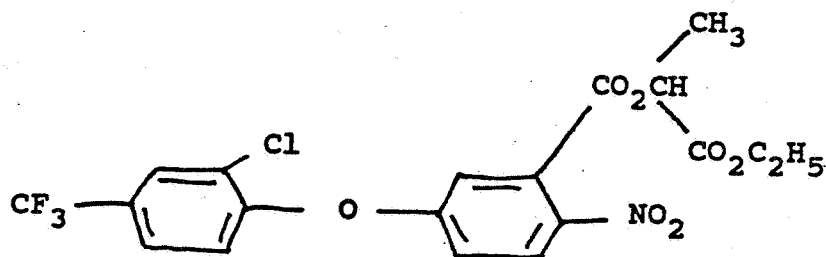
Oxyfluorfen



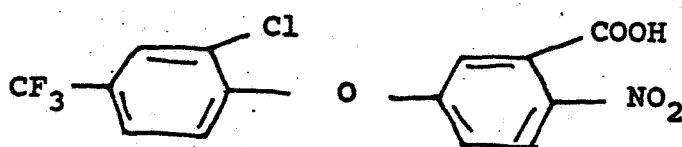
Fomesafen



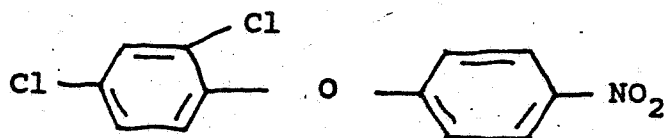
Lactofen



Acifluorfen



Nitrofen



F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Oxyfluorfen to be of importance in a weight-of-the-evidence determination of oncogenic potential.

1. Oxyfluorfen, when administered in the diet to male CD-1 strain mice at dietary doses of 0, 2, 20 and 200 ppm for 20 months, was associated with significant positive dose-related trends for liver adenoma, carcinoma and combined adenoma and/or carcinoma. There were no significant pairwise comparisons to untreated control.
2. The increased incidence of the liver tumors was found to be above the laboratory's historical control incidences for CD-1 mice.
3. There was no evidence for a reduction in the latency period for the time to liver tumor appearance in male mice.
4. There was no compound related increase in tumors observed in female mice or in male and female rats.
5. Both the mouse and rat studies were not considered to have been tested adequately at high enough doses to fully assess the oncogenic potential of Oxyfluorfen in either species.
6. Technical grade Oxyfluorfen was found positive for inducing gene mutations in the Salmonella and the mouse lymphoma assays suggesting it has mutagenic capability.
7. Oxyfluorfen was associated with adverse developmental effects in rats and rabbits and adverse reproductive effects in rats.
8. Oxyfluorfen is structurally related to four other diphenyl ether herbicides that have oncogenicity evidence associated with them. These chemicals include lactofen, acifluorfen (Blazer, Tackle), nitrofen and fomesafen. The primary neoplastic lesions induced by these chemicals include hepatocellular carcinomas and adenomas in mice, similar to that seen in the Oxyfluorfen study. These data provide strong support for the association of oncogenicity to this class of chemicals.

G. Classification of Oncogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

The Peer Review Committee unanimously concluded that the data available for Oxyfluorfen provided evidence to classify Oxyfluorfen as a Category C oncogen ("Possible Human Carcinogen"). This was based on the significant positive dose-related trends in liver adenomas, carcinomas and combined adenomas and/or carcinomas in male CD-1 mice. Supporting evidence included a strong association of oncogenicity with this class of chemical (diphenyl ether herbicides with nitro groups), some mutagenicity evidence and the appearance of increased carcinomas (although not significant by pairwise comparison).

The Committee concluded that quantification of oncogenic risk by Oxyfluorfen was not appropriate at this time. This was based on the data that included positive results in only one sex, one species, and no pairwise significant comparisons in tumor incidences. It was conjectured if higher doses in the mouse study had been used, more carcinomas may have appeared. However, it was felt that additional studies are not needed at this time. If additional studies were to become necessary, higher dosing would have to be used. A specific recommendation to perform an additional rat study was not made by the Committee at this time.